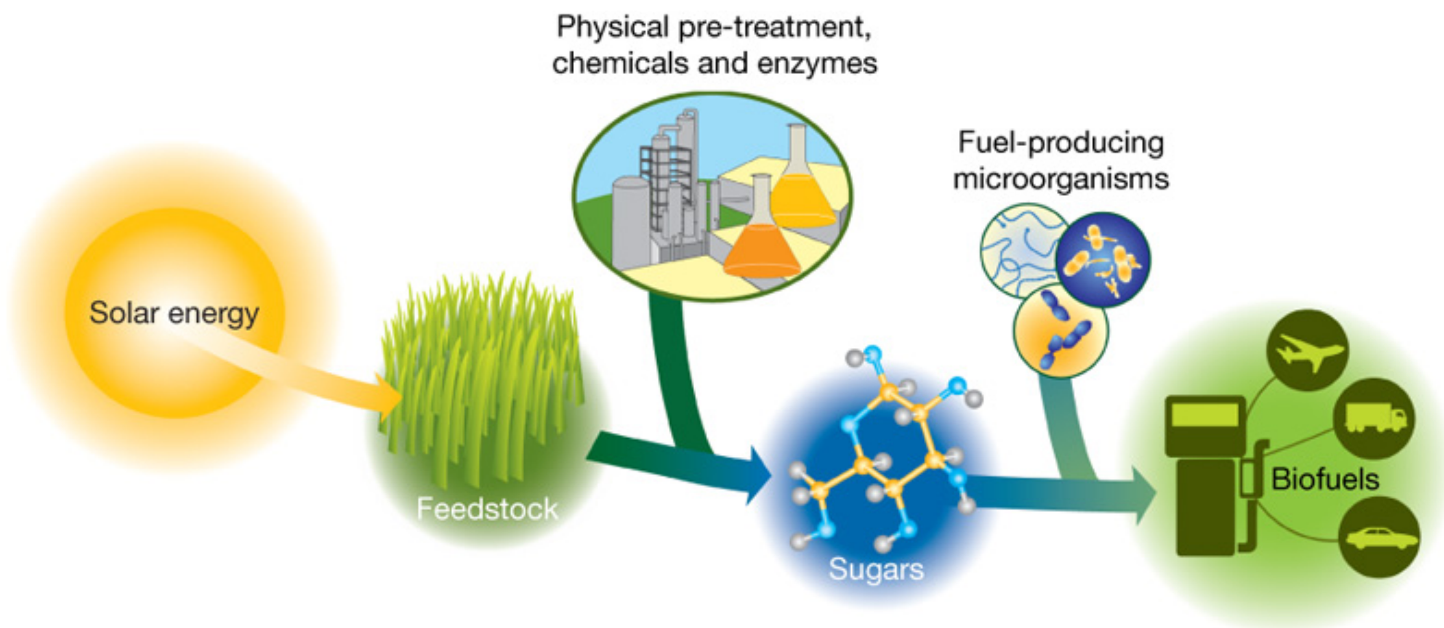
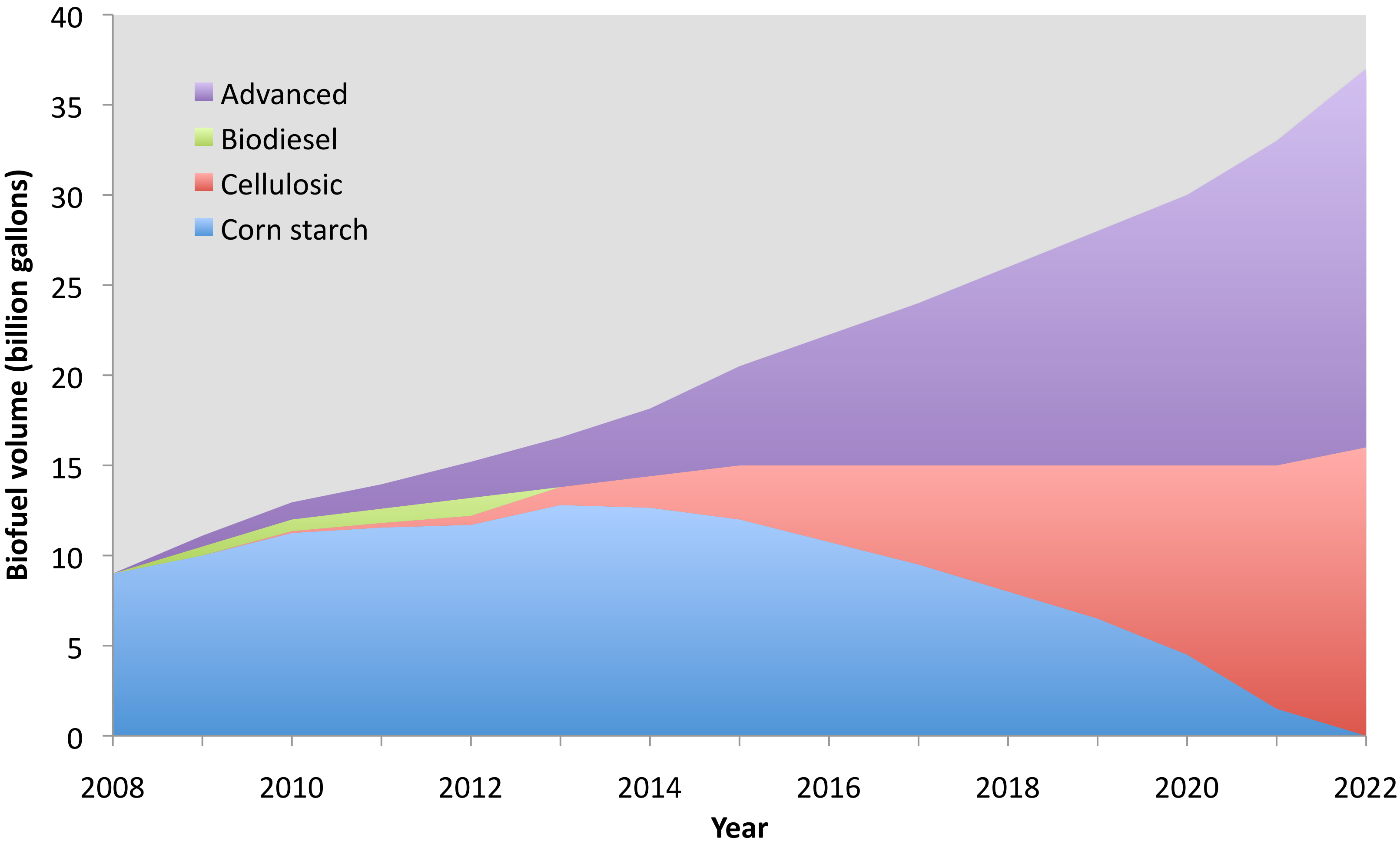
Systems biology of plant biofuel attributes



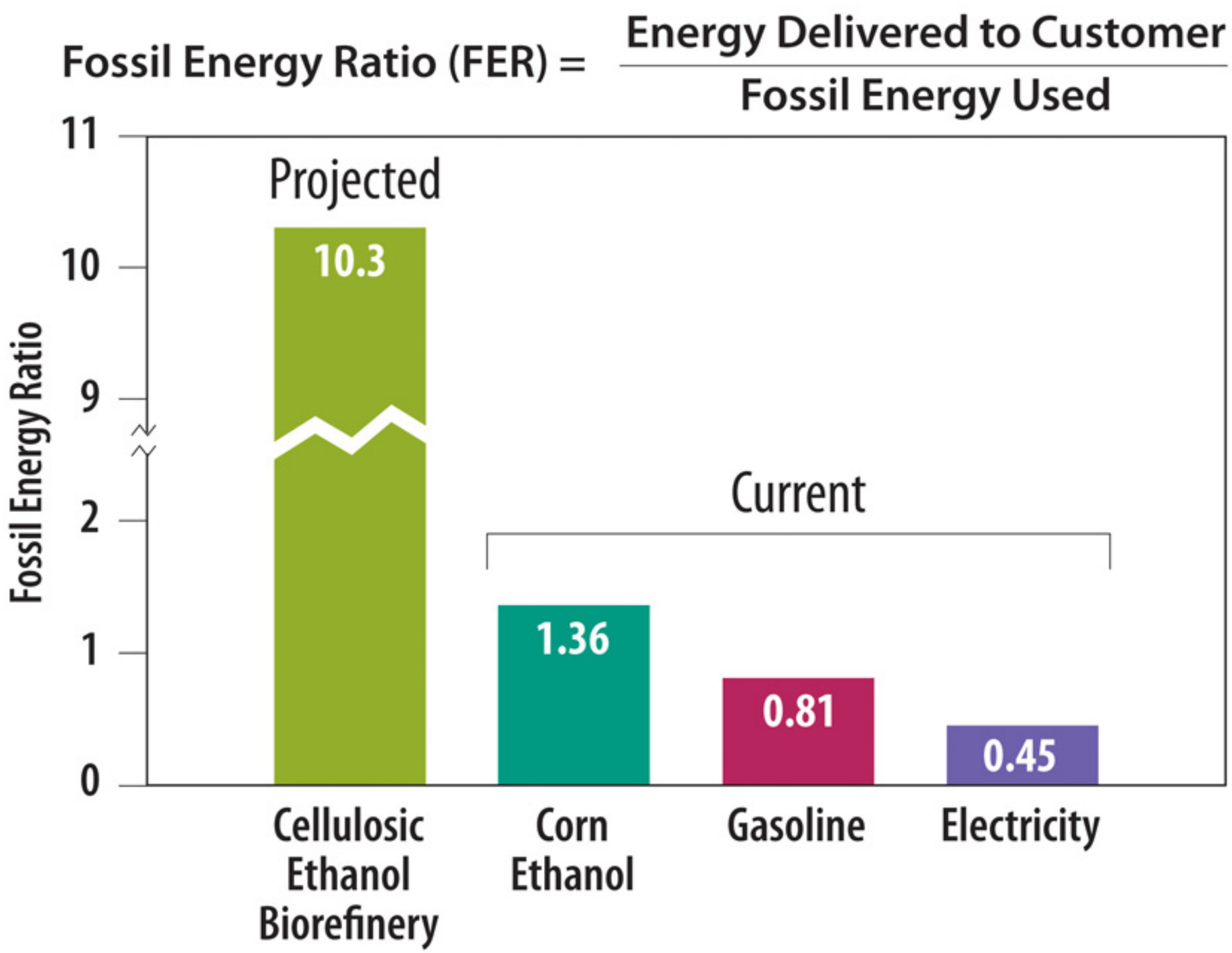
Sam Hazen
Biology Department
University of Massachusetts Amherst

National Renewable Fuel Standard Program

EPA revised requirements for Energy Independence and Security Act of 2007

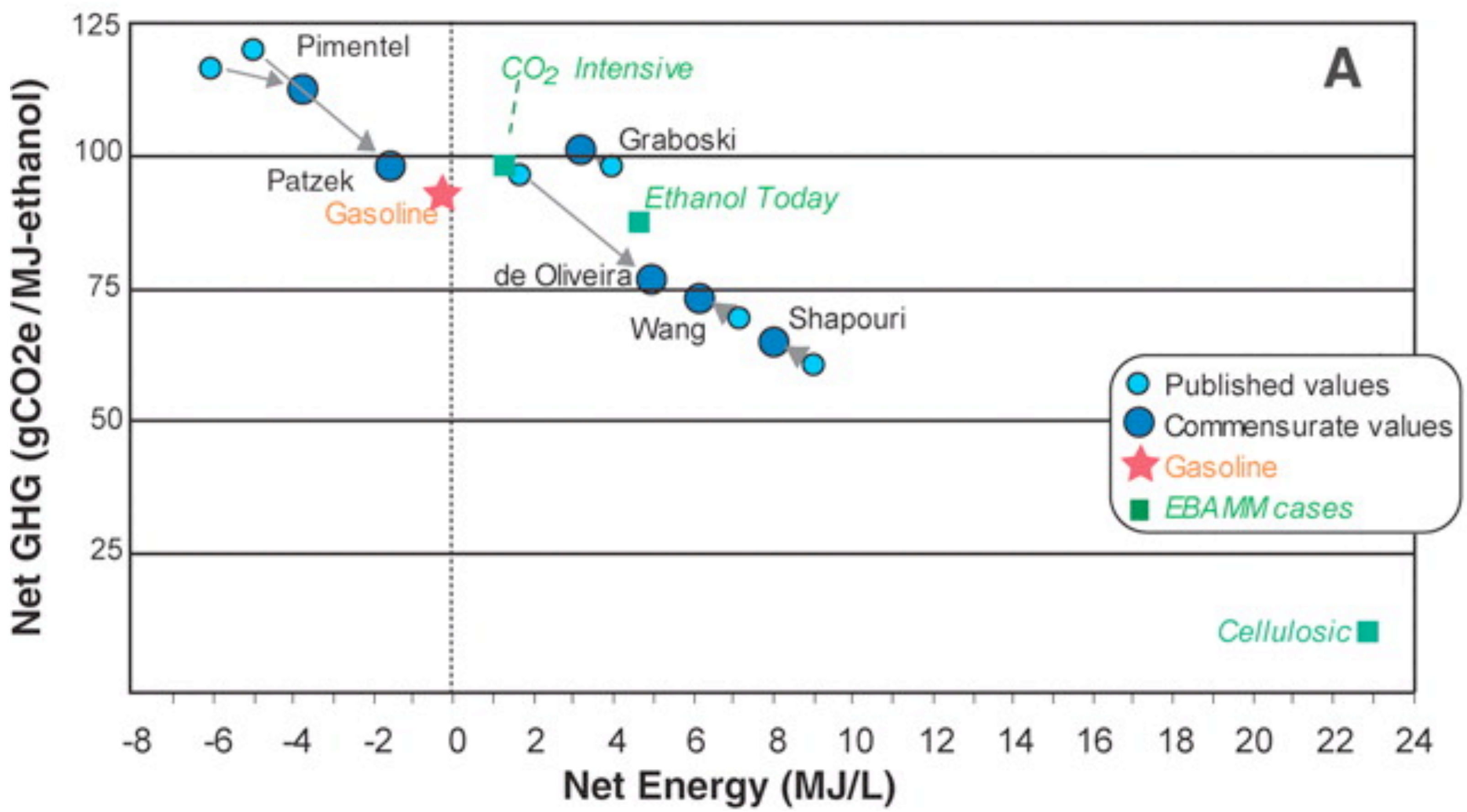


Comparison of energy yields with energy expenditures



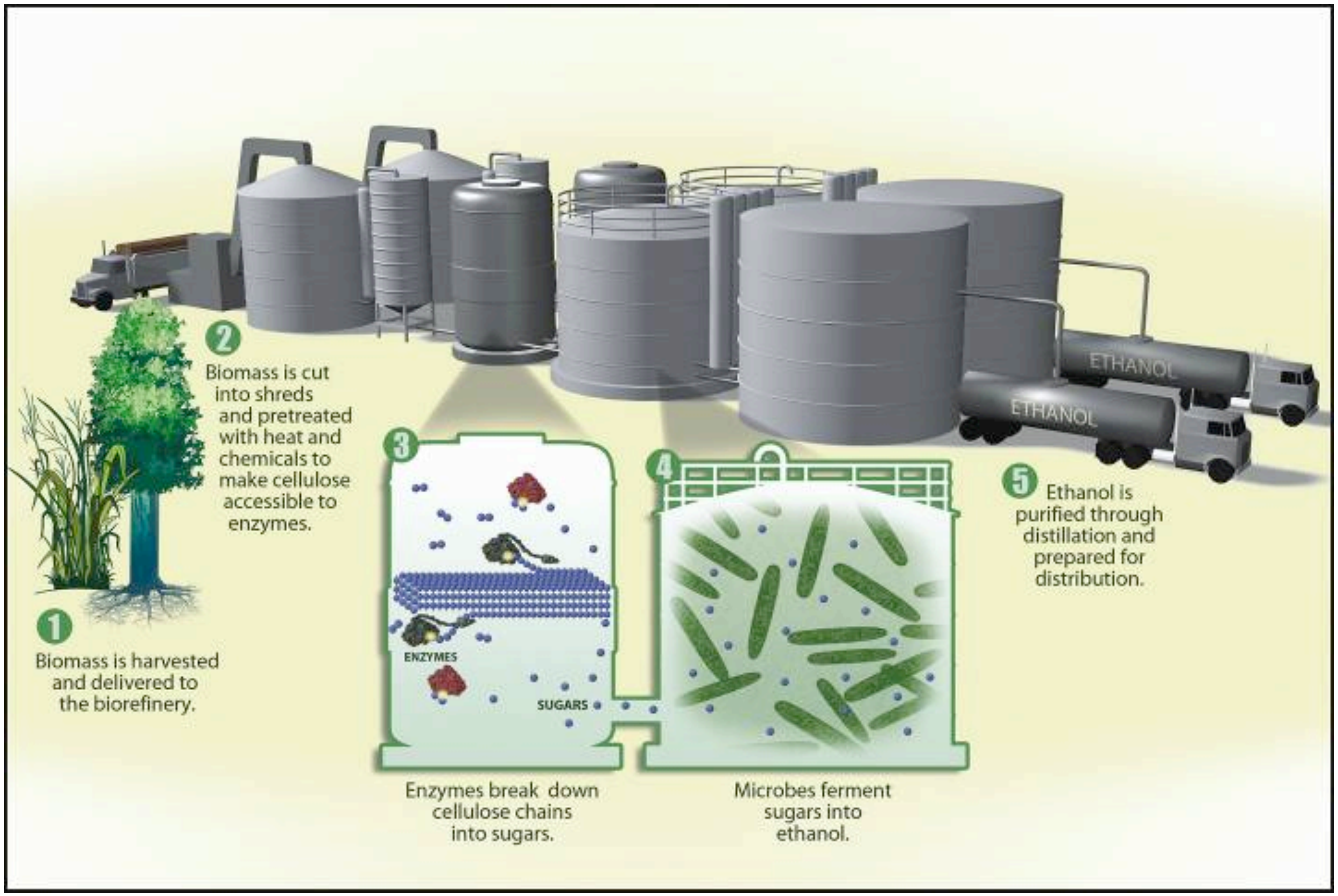
Based on the Argonne National Laboratory GREET model. DOE/SC/EE-0095, http://genomicscience.energy.gov/biofuels/

Net energy and greenhouse gases derived from bioethanol

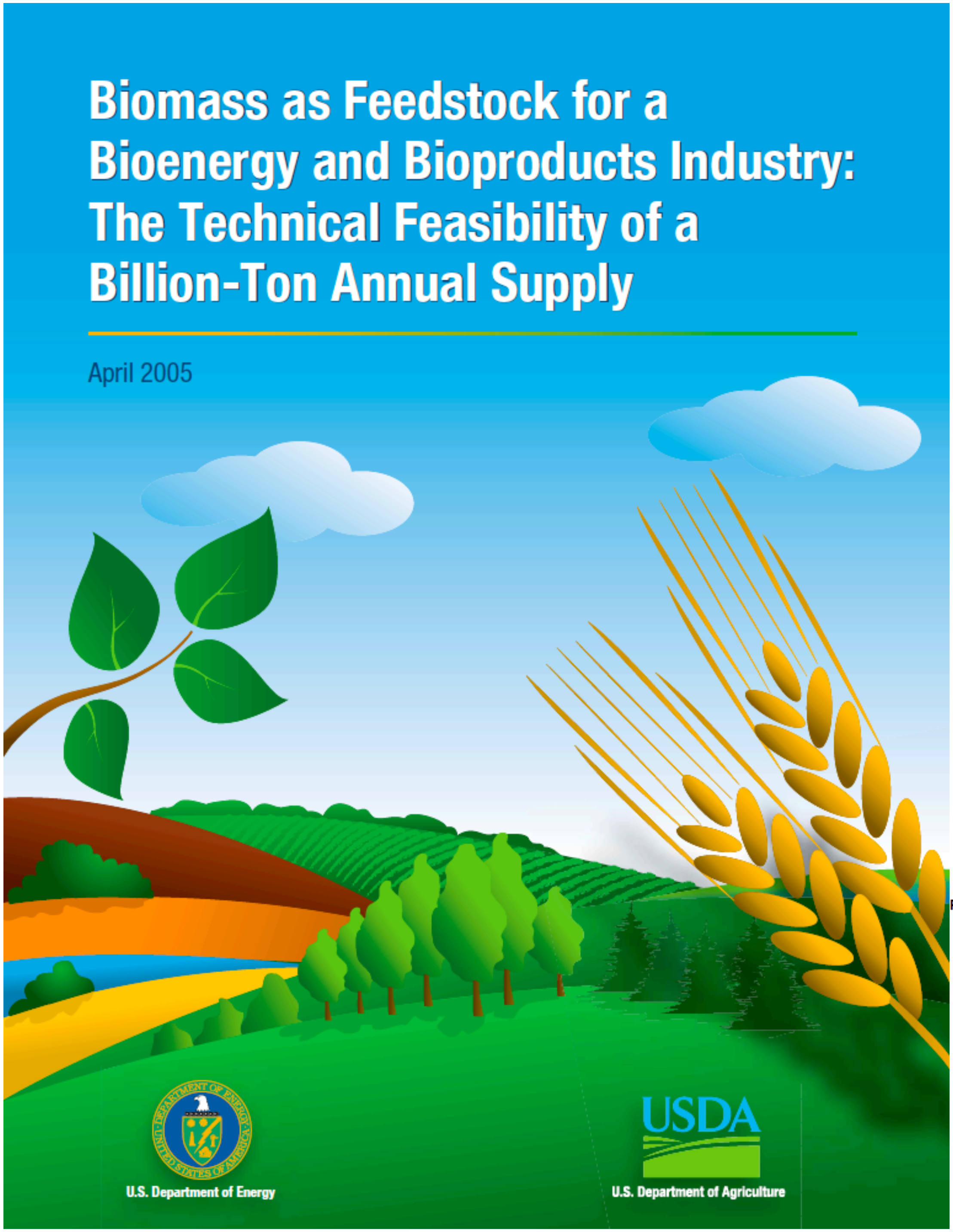


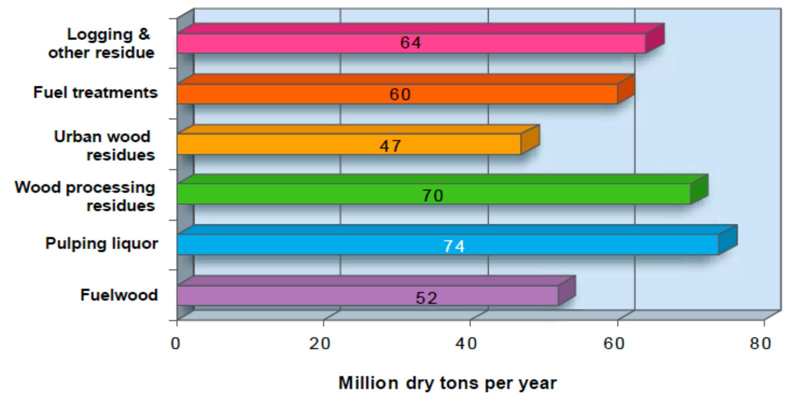
Alex Farrell, Dan Kammen, et. al., “Meta-analysis” of existing literature, Science 2006

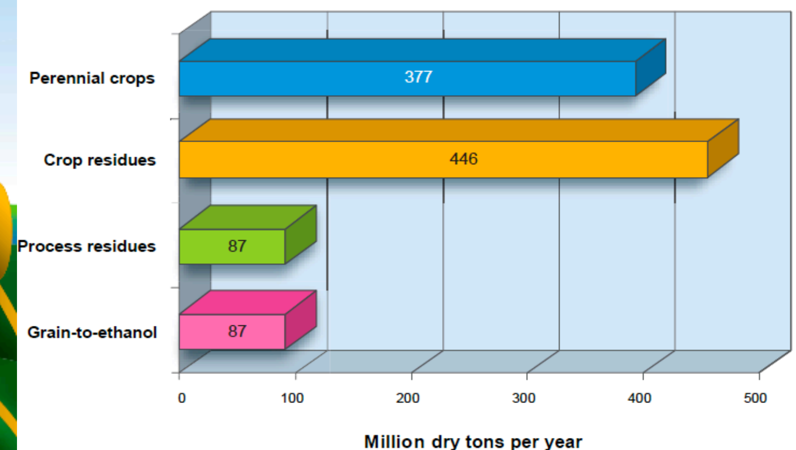
How cellulosic ethanol is made



Genome Management Information System, Oak Ridge National Laboratory


Biomass as Feedstock for a Bioenergy and Bioproducts Industry: The Technical Feasibility of a Billion-Ton Annual Supply
April 2005
U.S. Department of Energy
USDA
U.S. Department of Agriculture

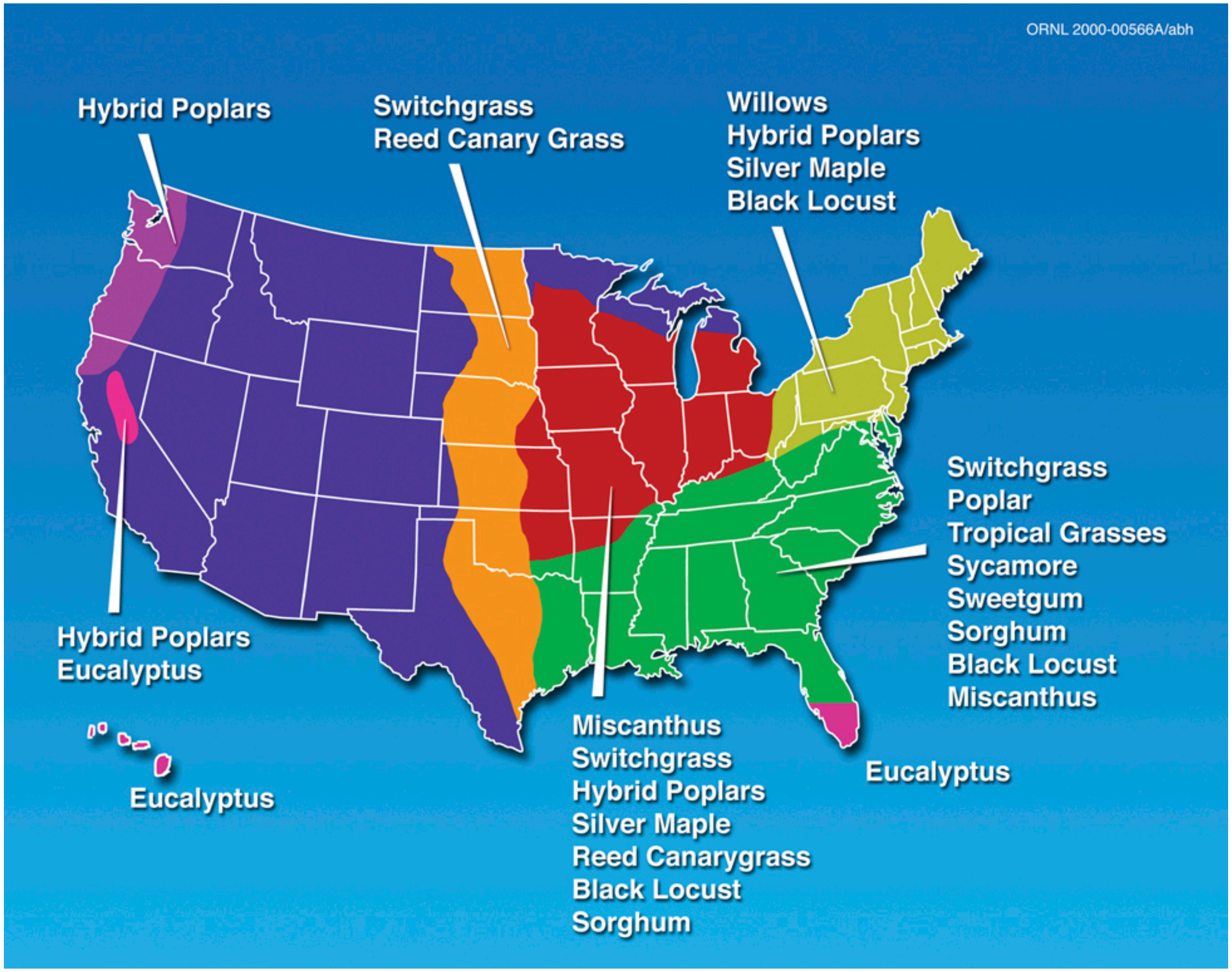

Logging & other residue
64
Fuel treatments
60
Urban wood residues
47
Wood processing residues
70
Pulping liquor
74
Fuelwood
52
0
20
40
60
80
Million dry tons per year


Perennial crops
377
Crop residues
446
Process residues
87
Grain-to-ethanol
87
0
100
200
300
400
500
Million dry tons per year

Crop residues

- >400M dry tons of crop residues
- Soil buffer from wind and rain
- Water holding capacity
- Nutrients
- Exacerbate cool moist conditions
- Harbor pathogens

Dedicated energy crops



Bioenergy Research Centers: An Overview of the Science, U.S. Department of Energy, February 2008. DOE/SC-0104

Miscanthus x giganteus and *Sorghum bicolor*

100M acres = 200B gal / year of ethanol

10 to 15 tons/acres

Courtesy of Steve Long, UIUC

26M acres = 377M tons

~7.5 tons/acre

Michael Kellett-Courtesy of Ceres, Inc

The ideal energy crop

- High yield
- Efficient solar capture
- Water and nutrient use efficiency
- Pest resistance
- Perennial growth habit
- Nutrient cycling
- Amenable to existing farm equipment
- Non-invasive
- End-use quality

The ideal energy crop

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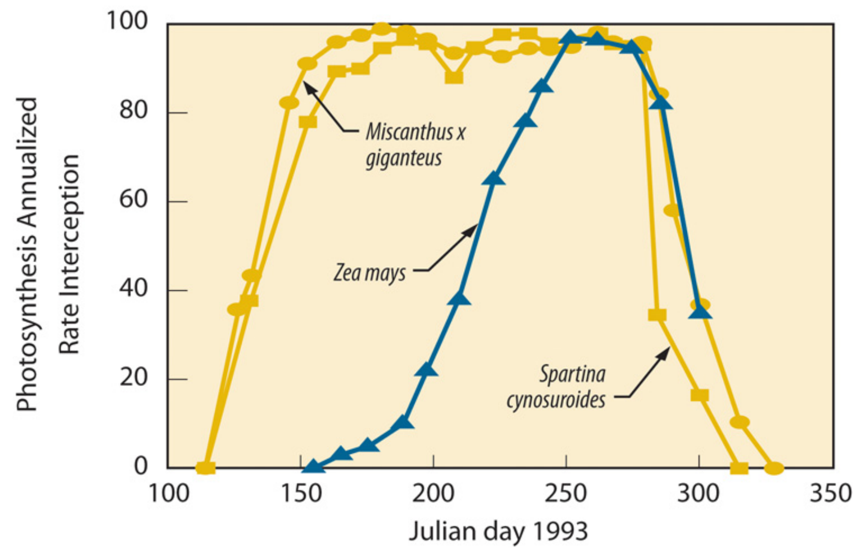
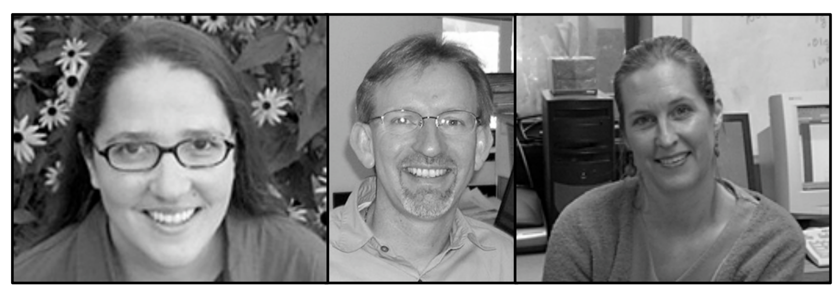


U.S. DOE. 2006. Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda, DOE/SC/EE-0095, U.S. Department of Energy Office of Science and Office of Energy Efficiency and Renewable Energy.



The ideal energy crop

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www.mendelbio.com

The ideal energy crop

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www.mendelbio.com

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Cox, T.S., J.D. Glover, D.L. Van Tassel, C.M. Cox, and L.R. DeHaan. (2006). Prospects for developing perennial grains. BioSciences, 56(8):649-659.

The ideal energy crop

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- Efficient solar capture
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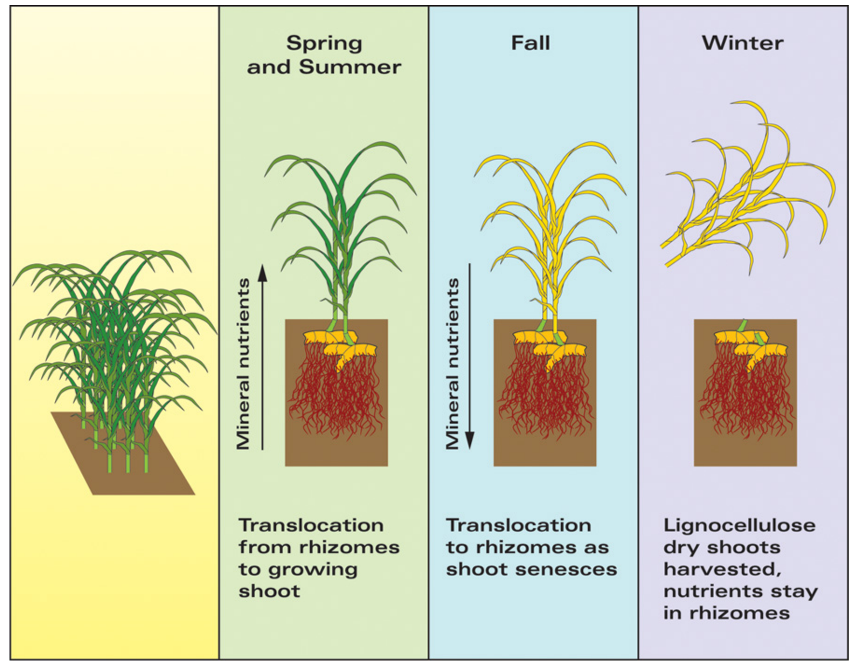

U.S. DOE. 2006. Breaking the Biological Barriers to Cellulosic Ethanol: A Joint Research Agenda, DOE/SC/EE-0095, U.S. Department of Energy Office of Science and Office of Energy Efficiency and Renewable Energy.

The ideal energy crop

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- Perennial growth habit
- Nutrient cycling
- Amenable to existing farm equipment
- Non-invasive
- End-use quality

www.reap-canada.com

The ideal energy crop

- High yield
- Efficient solar capture
- Water and nutrient use efficiency
- Pest resistance
- Perennial growth habit
- Nutrient cycling
- Amenable to existing farm equipment
- Non-invasive
- End-use quality

The ideal energy crop

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- Efficient solar capture
- Water and nutrient use efficiency
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- Perennial growth habit
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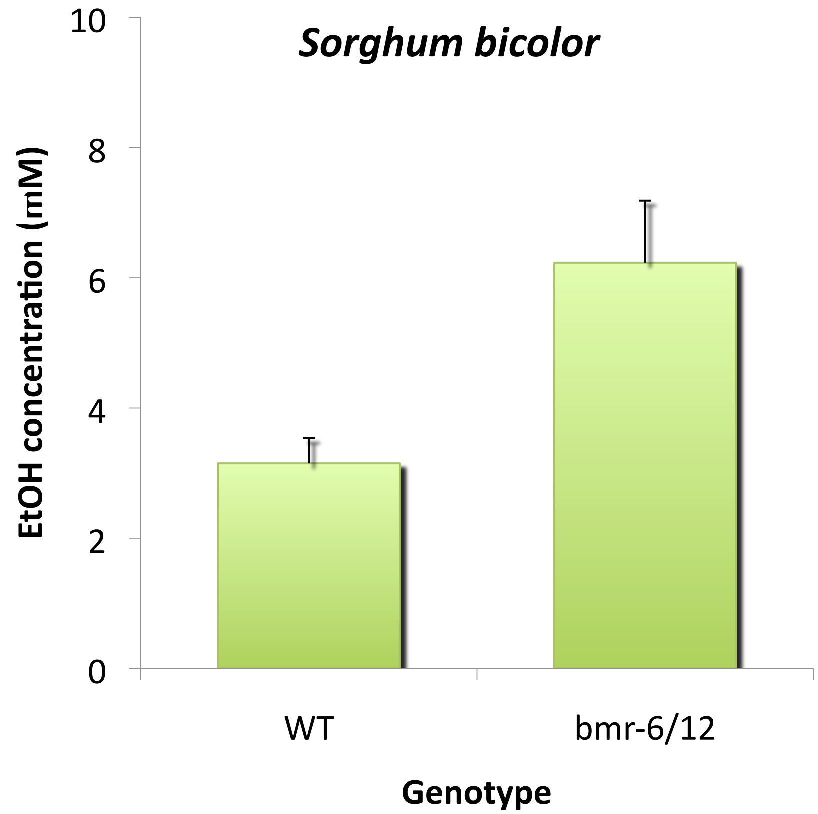
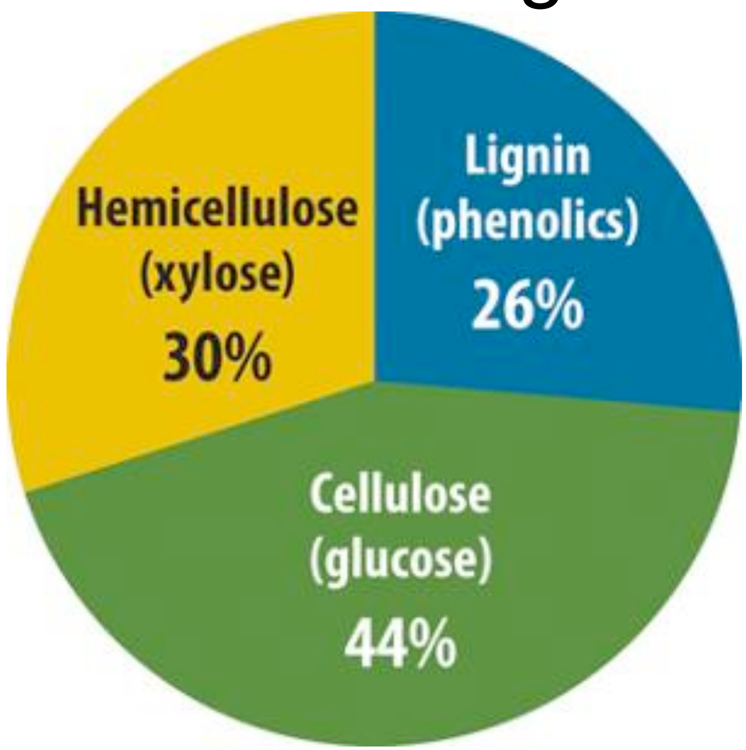
- End-use quality



Plant cell wall composition

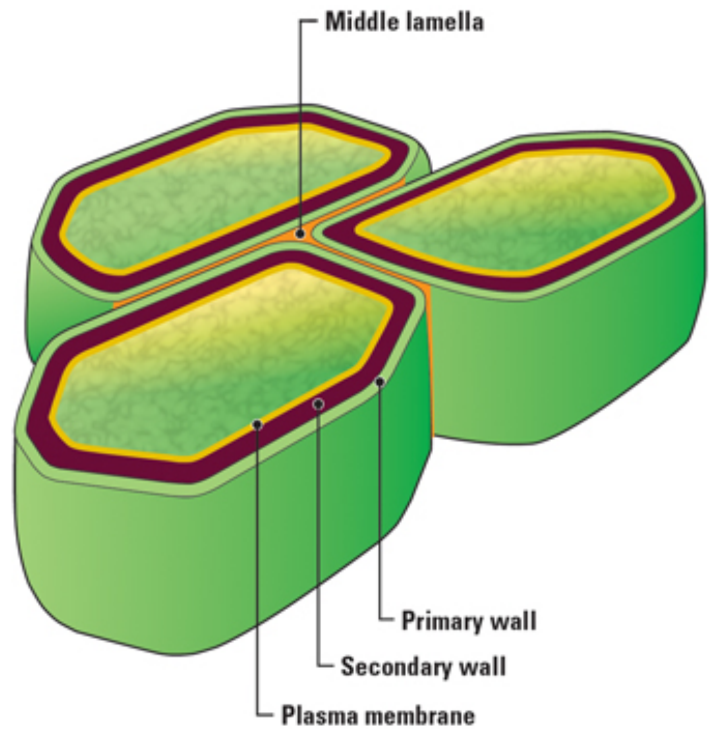
- Low cost source of mixed sugars



Genome Management Information System, Oak Ridge National Laboratory

Plant cell wall composition

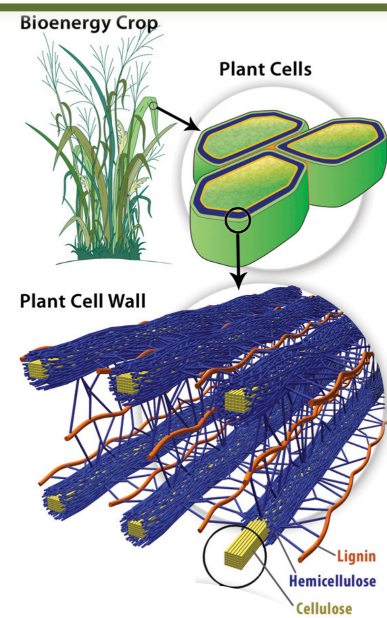
- A defining feature of plants is a **cell wall** exquisitely designed to support plant structure and resist biological and chemical assault.



Genomics:GTL Transforming Cellulosic Biomass, U.S. Department of Energy Office of Science and Office of Energy Efficiency and Renewable Energy, June 2006

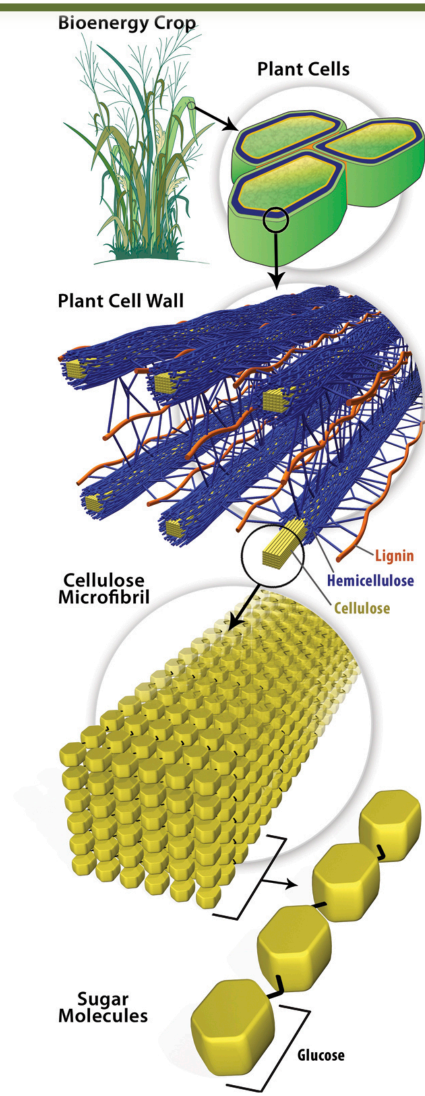
Plant cell wall composition

- Within the plant cell wall are chains of **cellulose** molecules that associate with **hemicellulose** and **lignin** to form linear structures of high tensile strength.



Plant cell wall composition

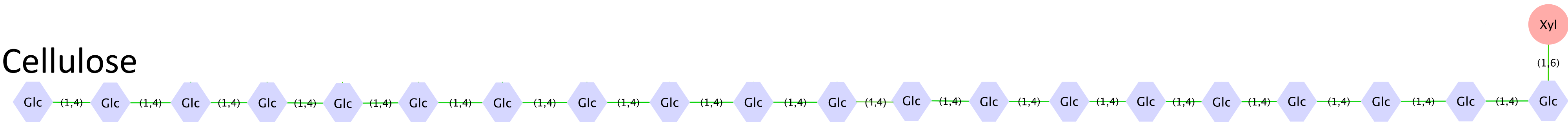
- The simple sugars that make up these polymers can potentially provide low cost feedstock for **biofuel** production.



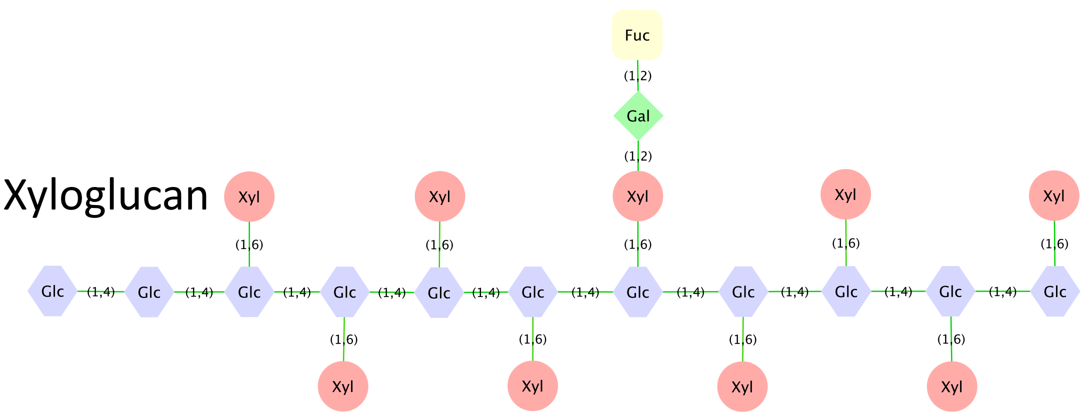
Bioenergy Research Centers: An Overview of the Science, U.S. Department of Energy, July 2009. DOE/SC-0116

Hemicelluloses are shorter heterogeneous polymers

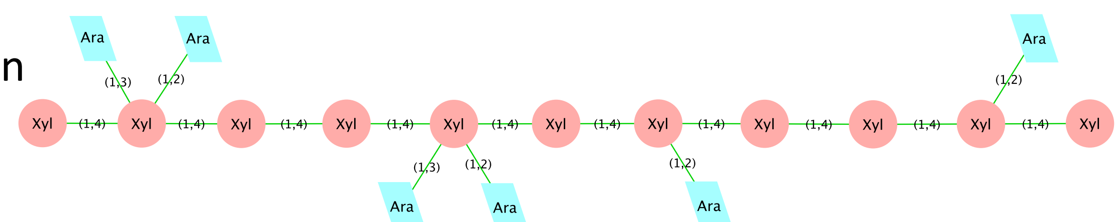
Cellulose



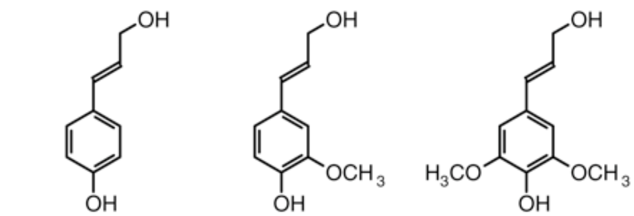
Xyloglucan



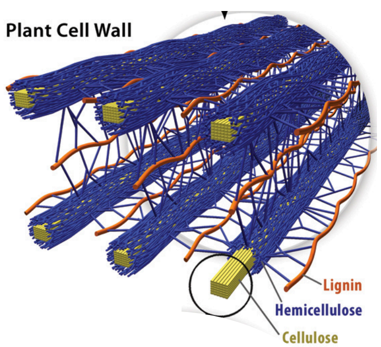
Arabinoxylan

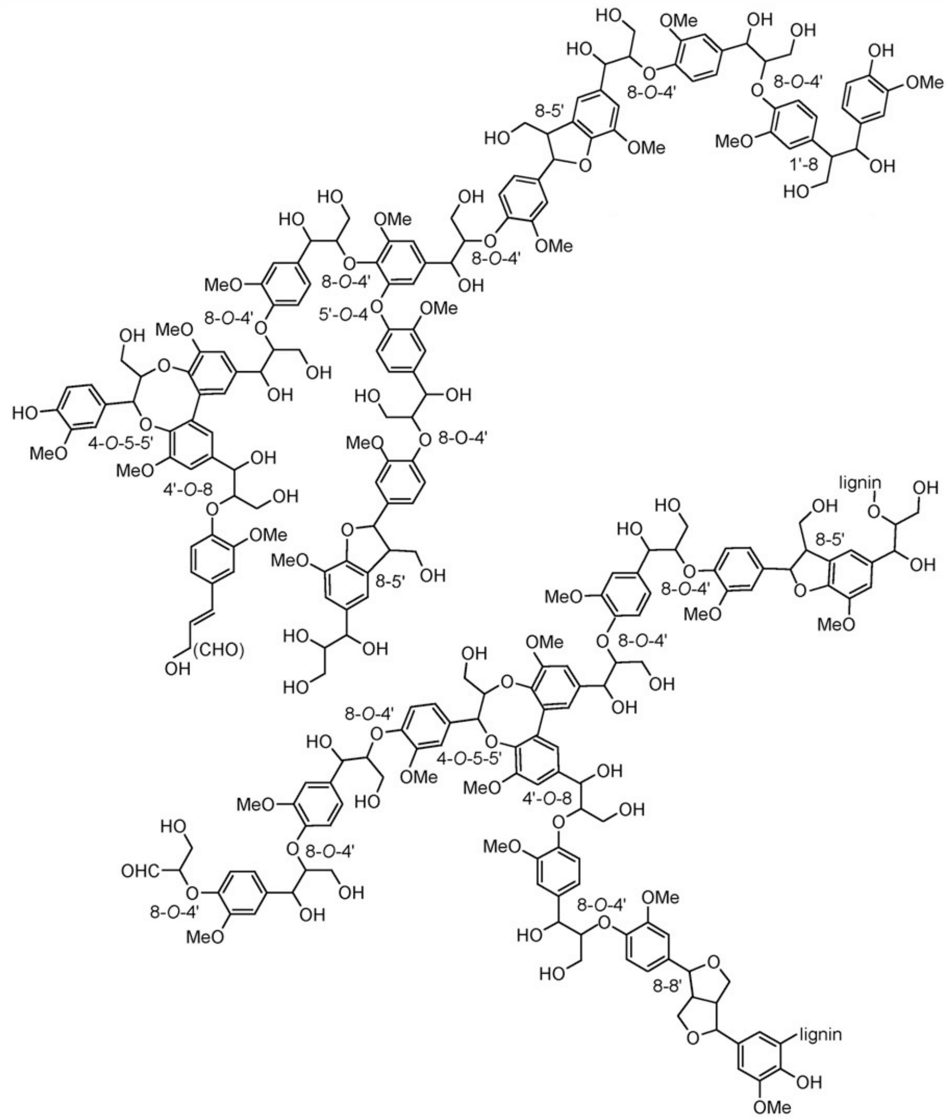


Lignin is a cross-linked phenolic polymer that interferes with the degradation of cell walls



p-coumaryl alcohol　　coniferyl alcohol　　sinapyl alcohol





The angiosperm lignin biosynthetic pathway

ring modification →

side chain modification ↓

phenylalanine

PAL

cinnamate — C4H — *p*-coumarate

4CL

p-coumaroyl CoA

HCT

p-coumaroyl shikimate — C3H — caffeoyl shikimate

HCT

caffeoyl CoA — CCoAOMT — feruloyl CoA

CCR

coniferyl aldehyde — 5-OH coniferyl aldehyde — sinapyl aldehyde

CAD

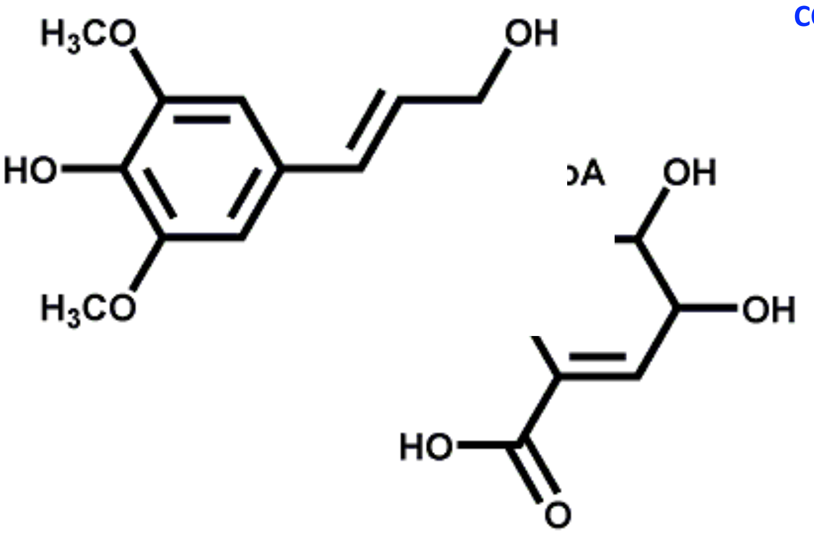
coniferyl alcohol — F5H — 5-OH coniferyl alcohol — COMT — sinapyl alcohol

PER/LAC

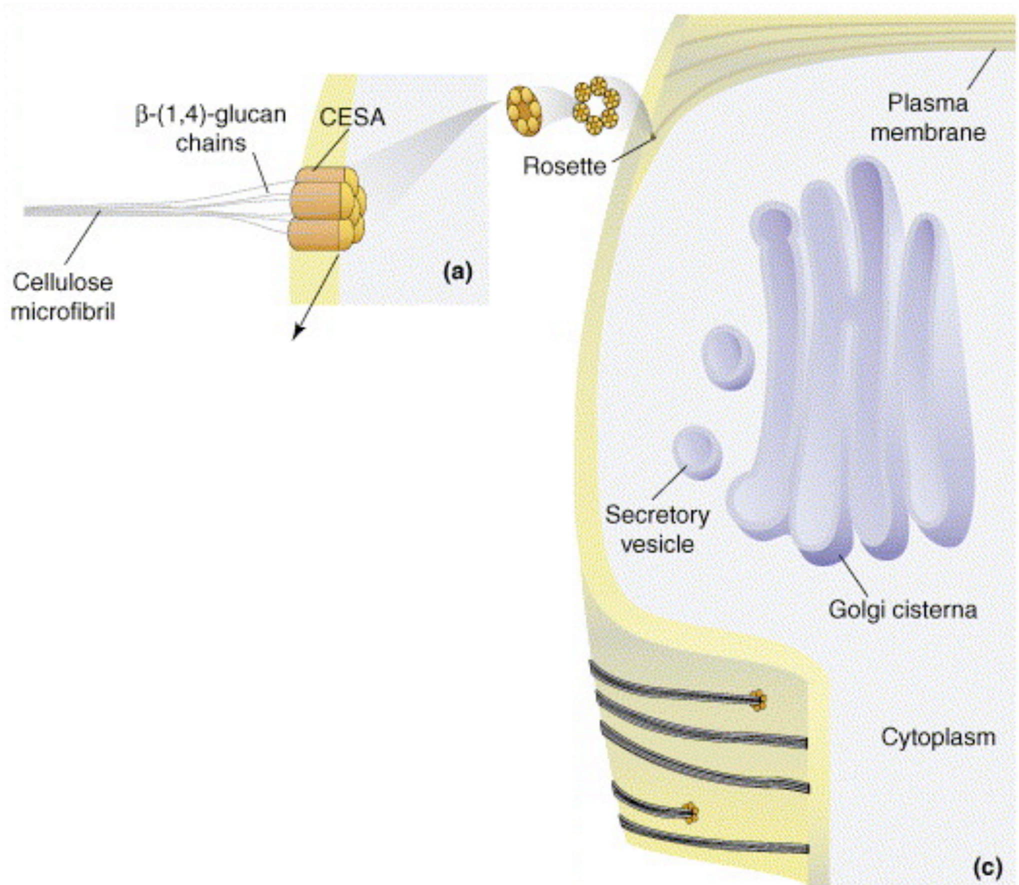
guaiacyl lignin

PER/LAC

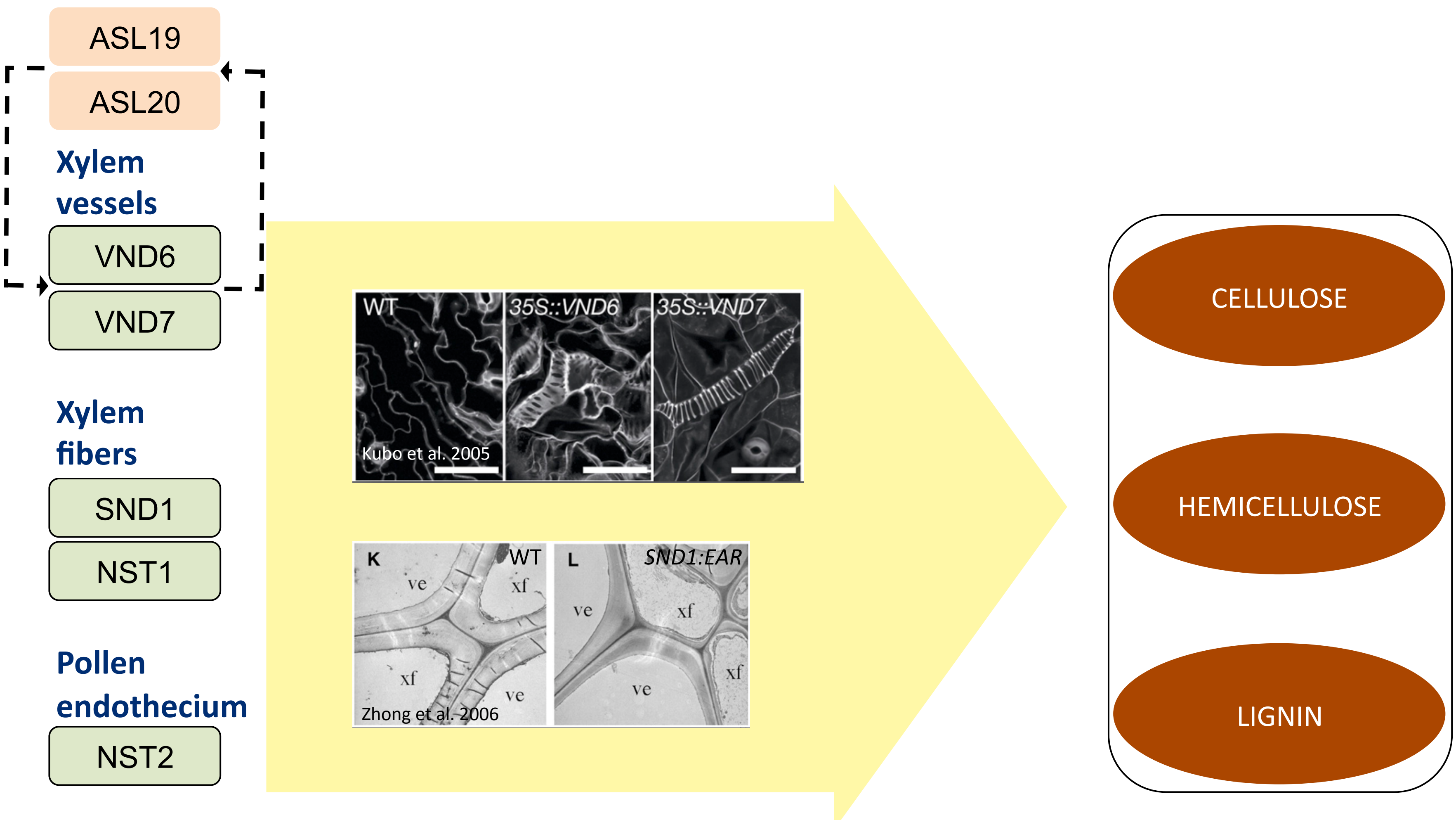
syringyl lignin



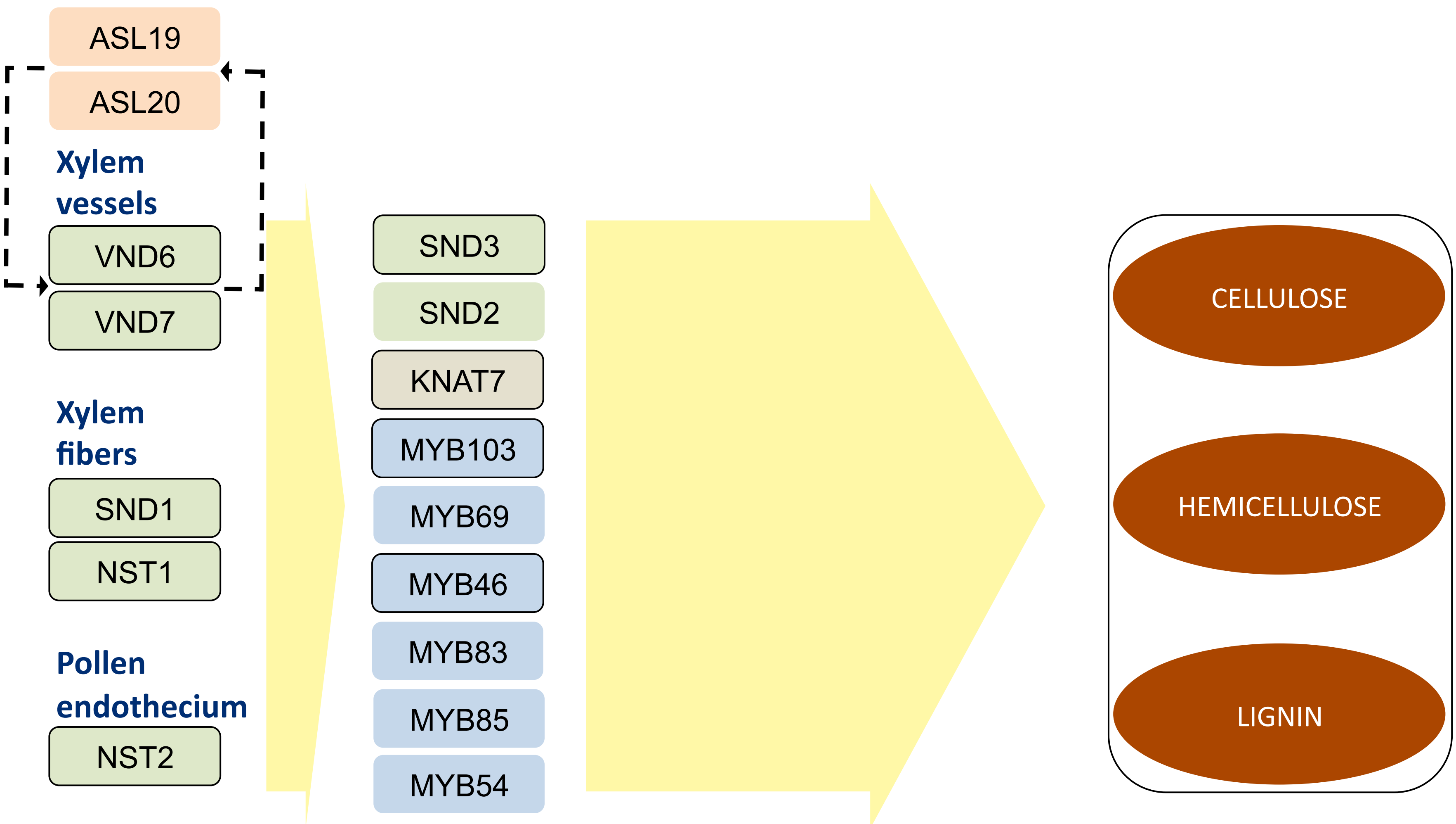
Cellulose biosynthetic enzymes



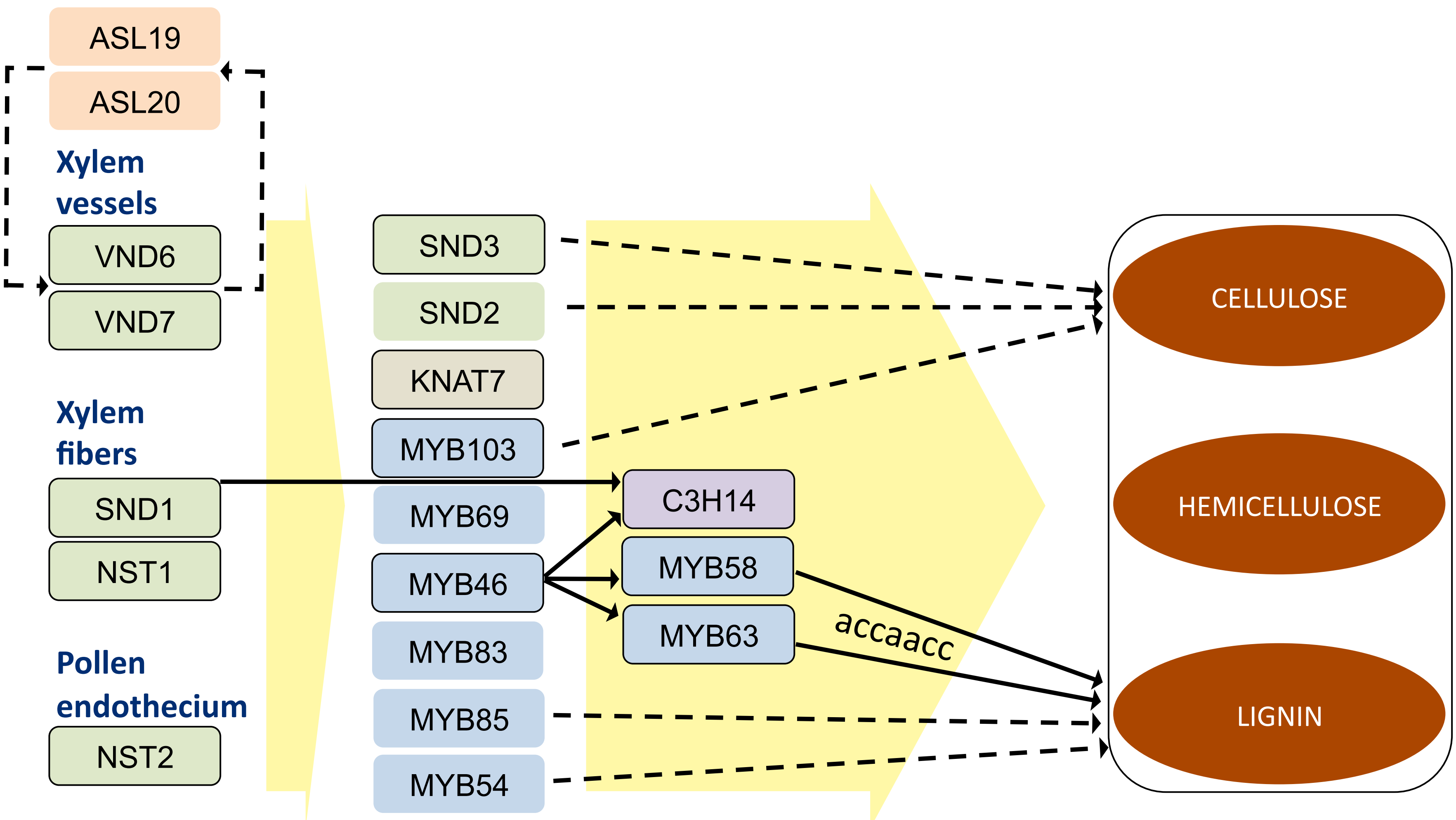
Cell wall regulatory network



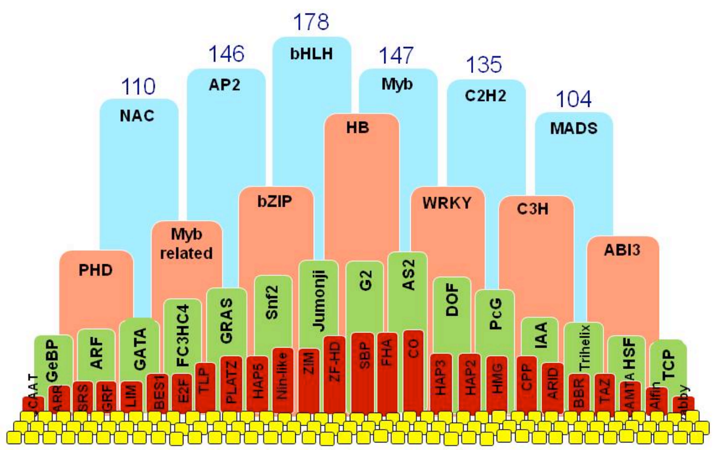
Cell wall regulatory network



Cell wall regulatory network



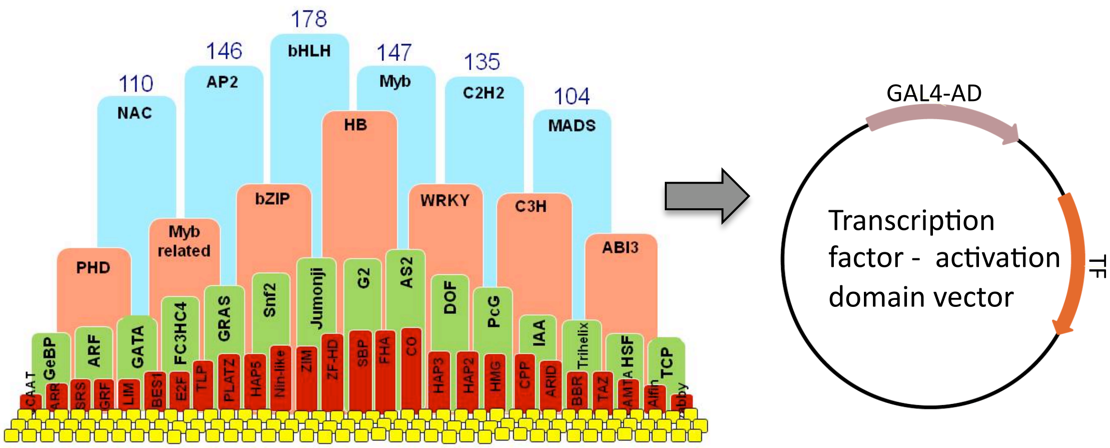
Regulatory network discovery



Library of ~1800 plant transcription factors (TFs)

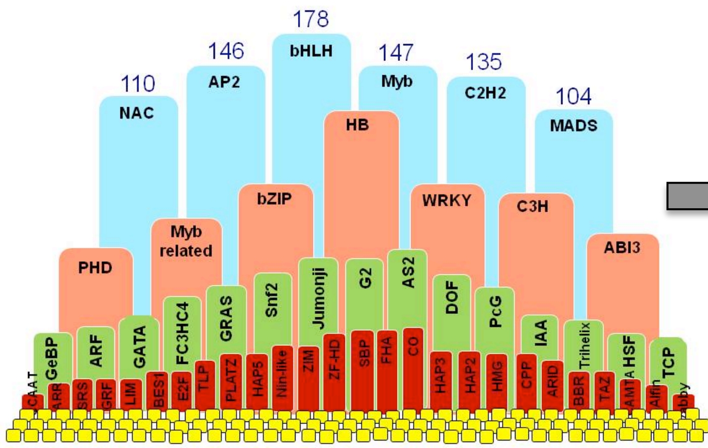
Bind to specific DNA sequences and either promote or block transcription

Regulatory network discovery



Library of ~1800 plant transcription factors (TFs)

Regulatory network discovery



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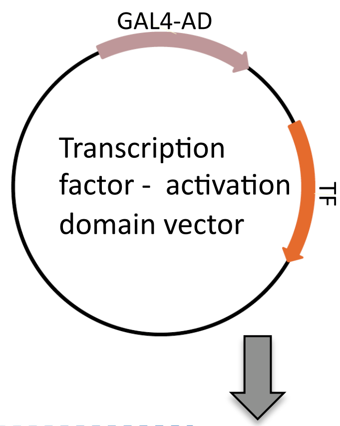
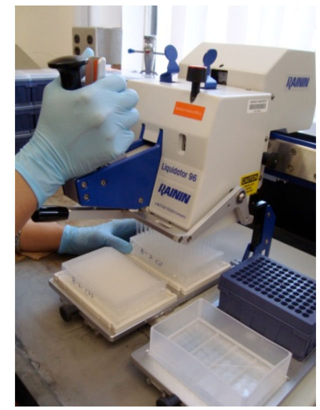
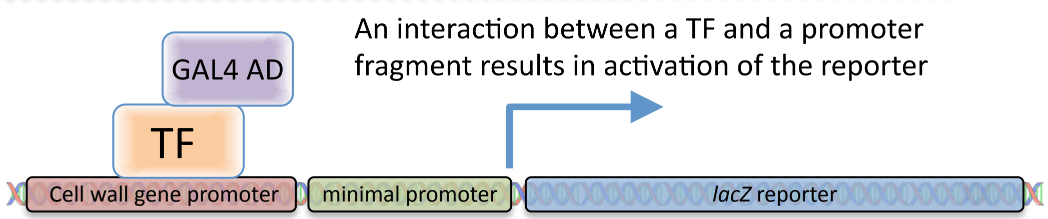
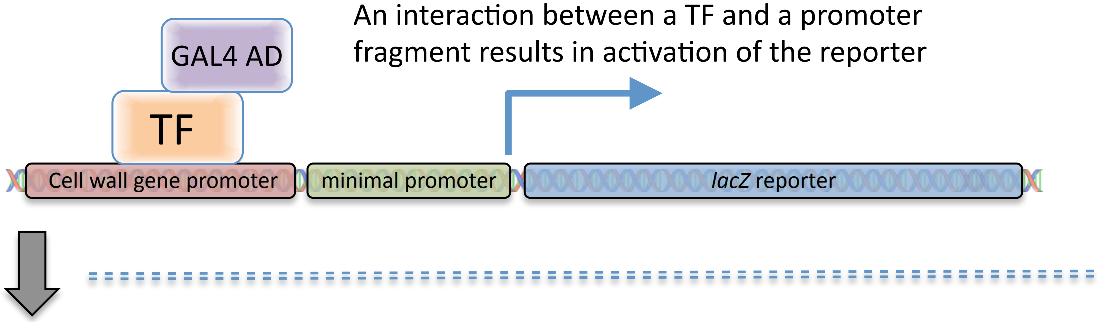


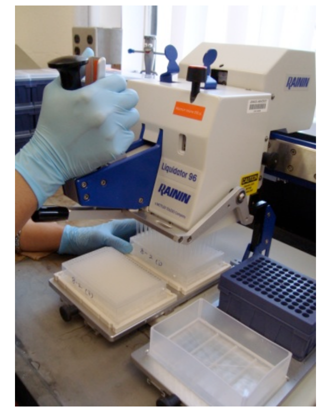


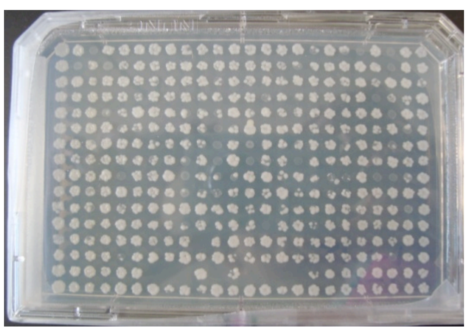


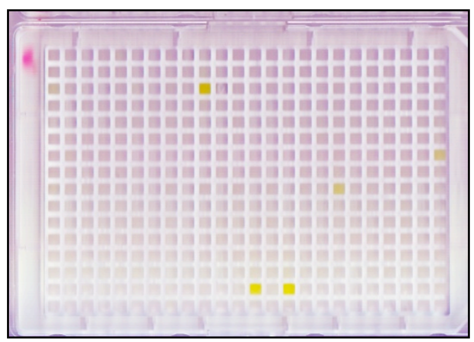
Regulatory network discovery

An interaction between a TF and a promoter fragment results in activation of the reporter



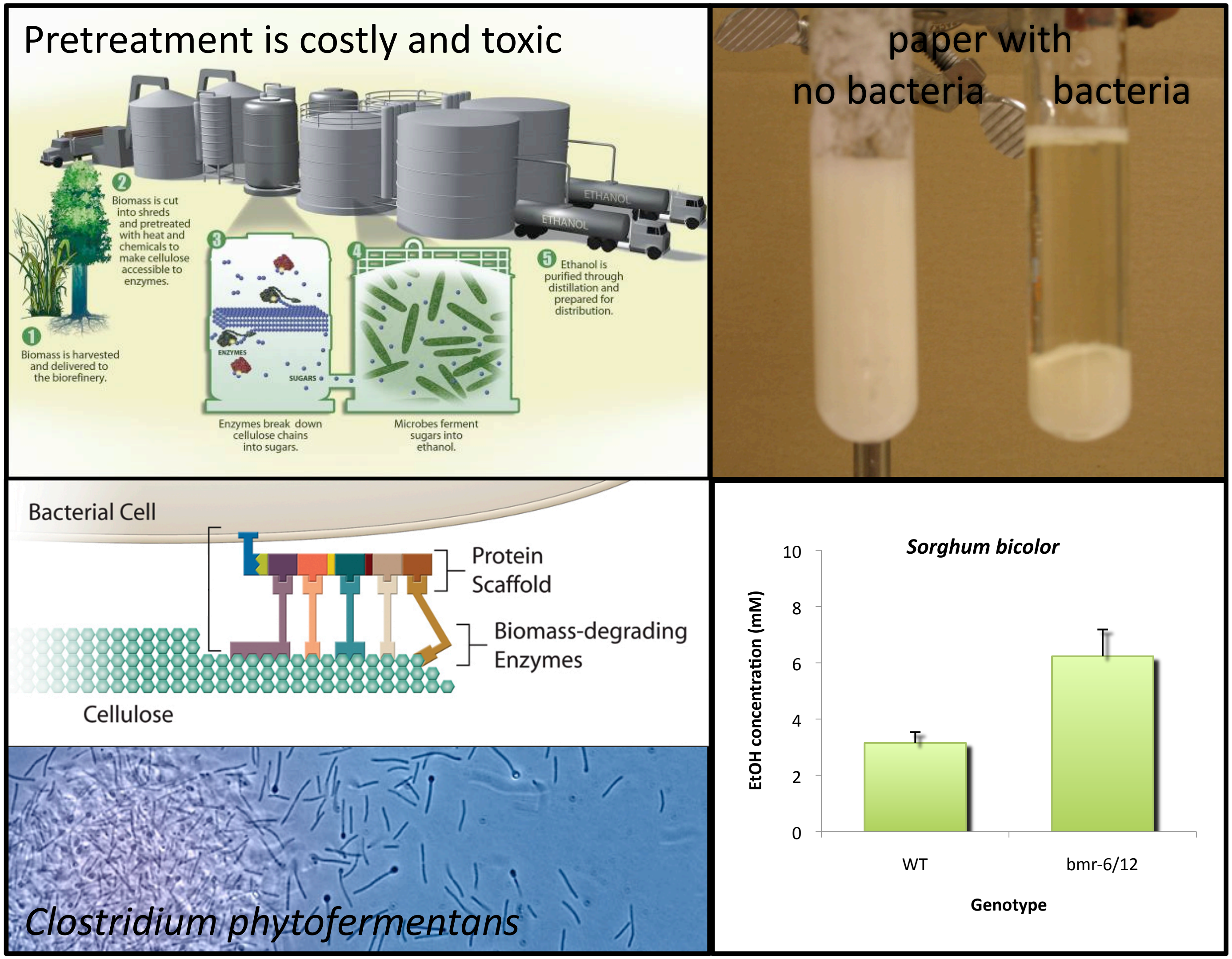






Protein-DNA interactions are measured quantitatively for each TF. Actual results from a screen of a *CELLULOSE SYNTHASE A* promoter. These candidates now become targets for functional characterization of their role in cell wall biosynthesis

Translational bioassay for plant feedstock properties



PRODUCT SNAPSHOT: MISCANTHUS

Plants can grow 12 feet high or more

Stands re-grow each spring and can last 10+ years

Long harvest window

Once established, requires little or no fertilizer or weed control

There are 37,000 acres of miscanthus planted in the United Kingdom

Miscanthus-to-biofuels is carbon neutral

Target Market

PRODUCT SNAPSHOT: SWITCHGRASS

Switchgrass can grow 9-feet high with roots just as deep.

Each acre of switchgrass can sequester the equivalent of 5 tons of CO_2 each year.

Stands re-grow each spring.

Switchgrass provides ideal cover for ground-nesting birds.

Target Market

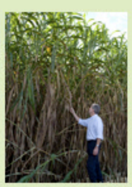
PRODUCT SNAPSHOT: SORGHUM

Naturally drought tolerant; high water-use efficiency

Plants can approach 20 feet high under favorable conditions.

Well established hybridization systems

Fast-growing annual energy crop.



Target Market

PRODUCT SNAPSHOT: ENERGYCANE

Energycane is a perennial; stands lasts several years

Traditional breeding and selection of cane is complex and slow since viable seeds are seldom produced

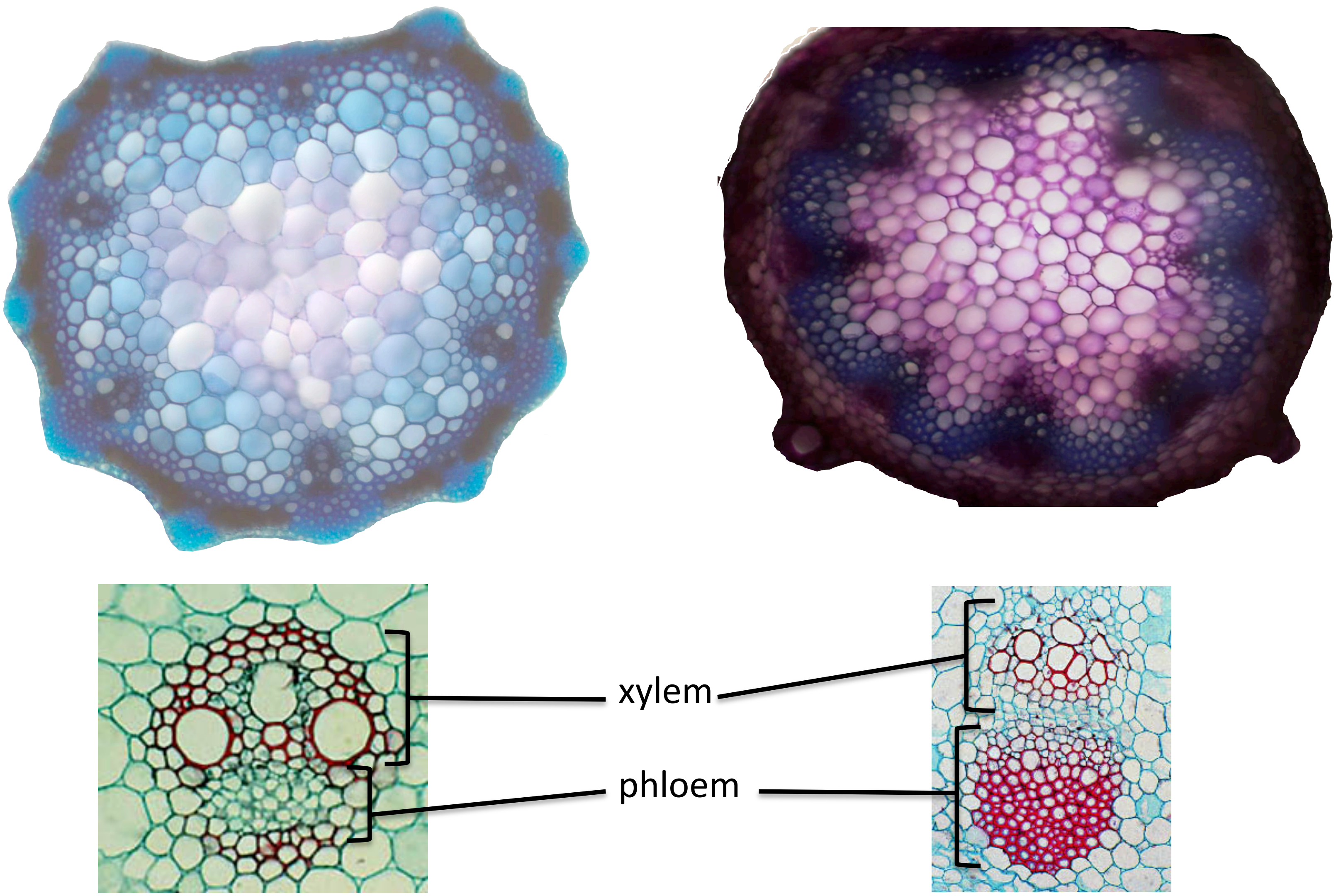
Canes are reproduced through cuttings; not through seed

Energycane will likely be grown in tropical or subtropical regions

Target Market

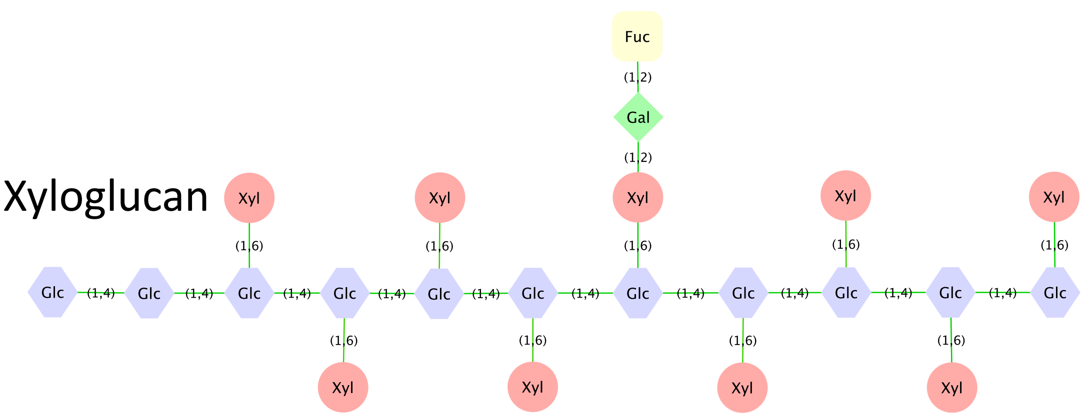
c e r e s

Monocots and dicots have different cell types and morphology

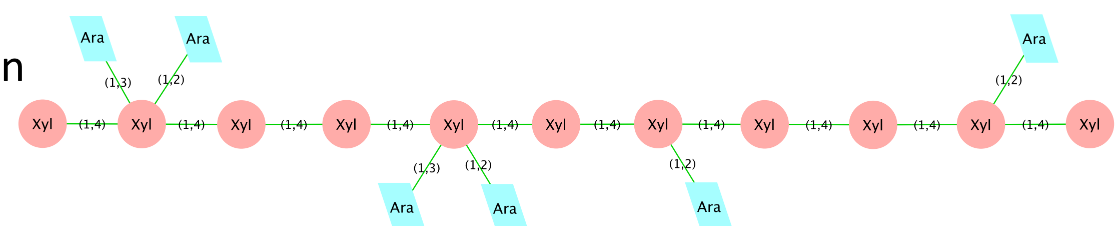


Monocots and dicot cell walls are compositionly different

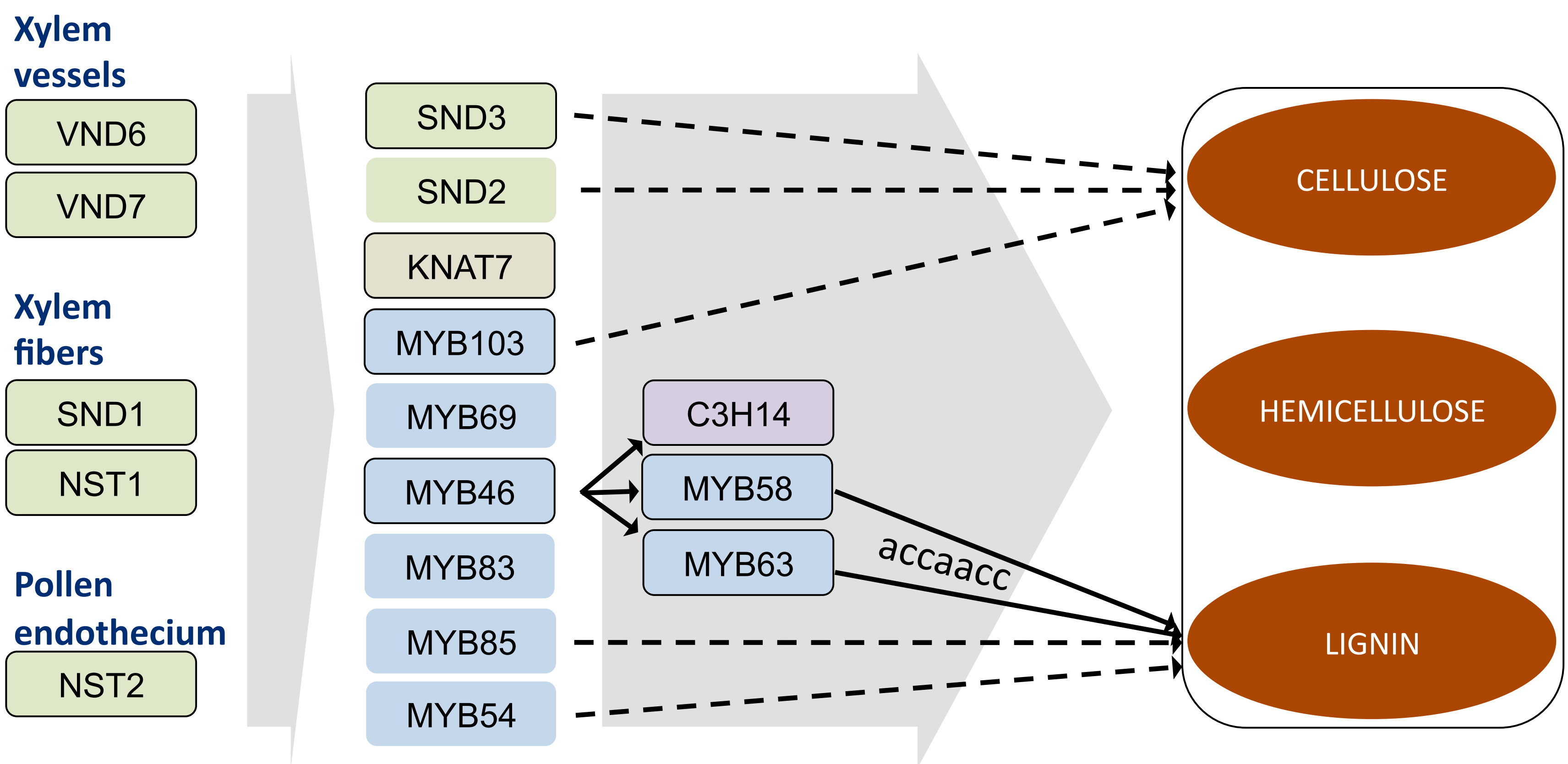
Xyloglucan



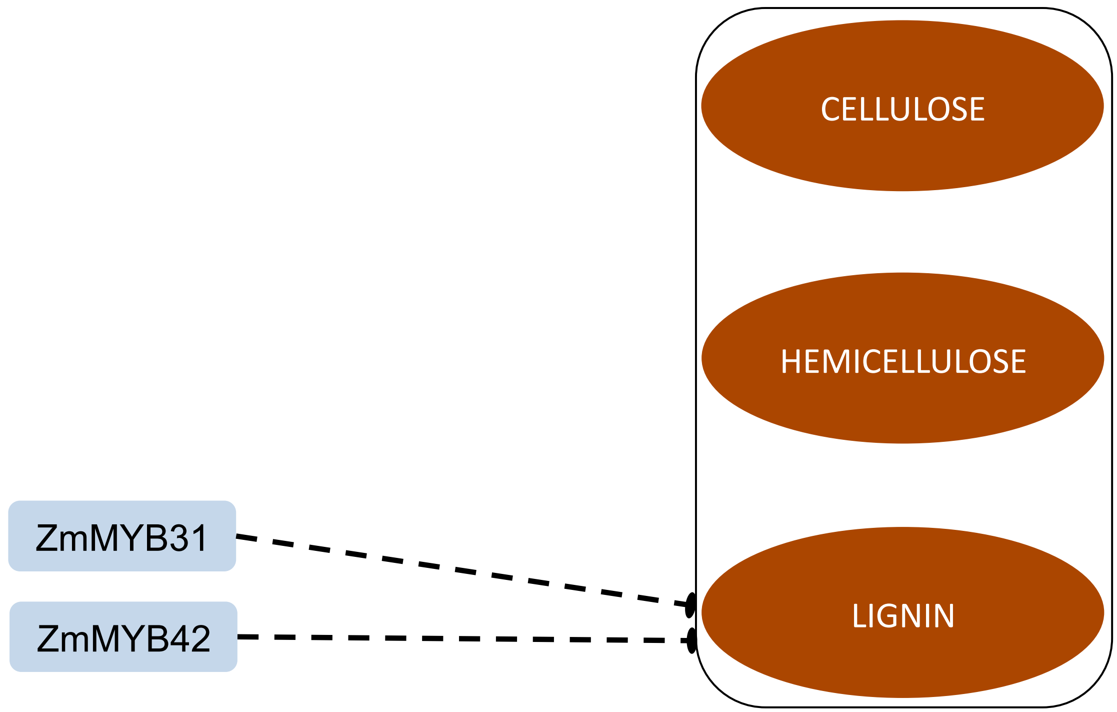
Arabinoxylan



Cell wall regulatory network

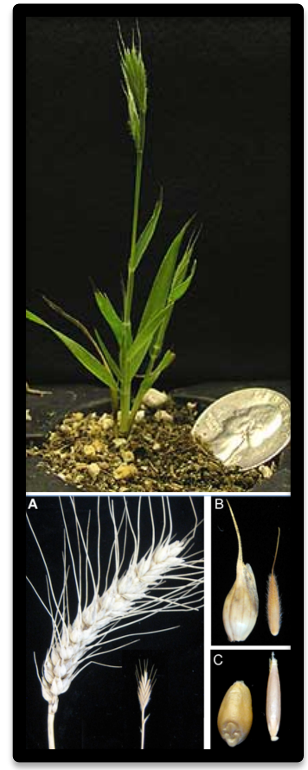


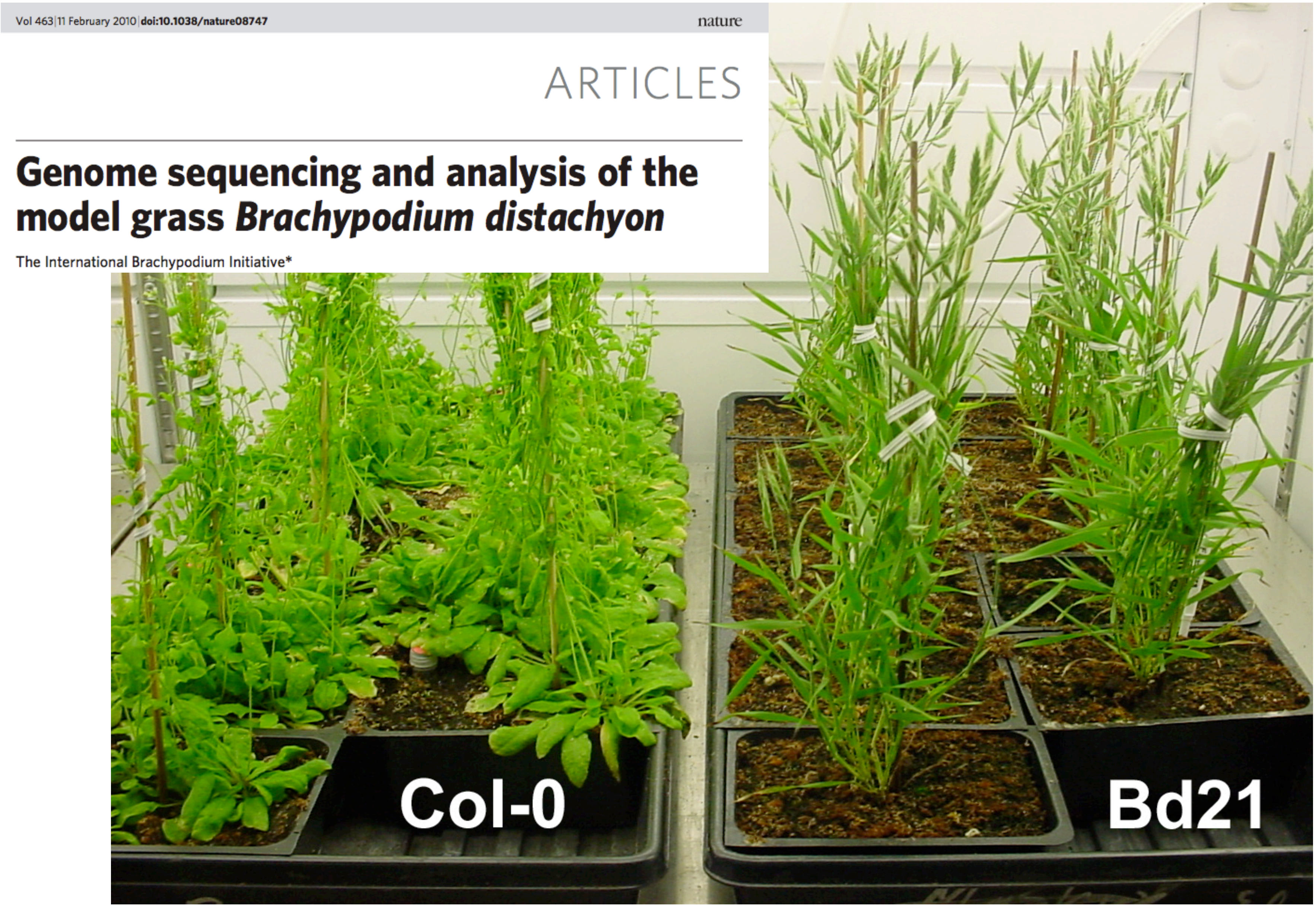
Cell wall regulatory network



Science with crops

System attributes	Crops	brachy
Life cycle	Months to years	Four weeks
Stature	3 to 15ft	Small
Fecundity	None to high	Decent
Genome complexity	Moderate to baffling	Simple
Transformation	Challenging to impossible	Simple
Genetic resources	None to few	Extraordinary





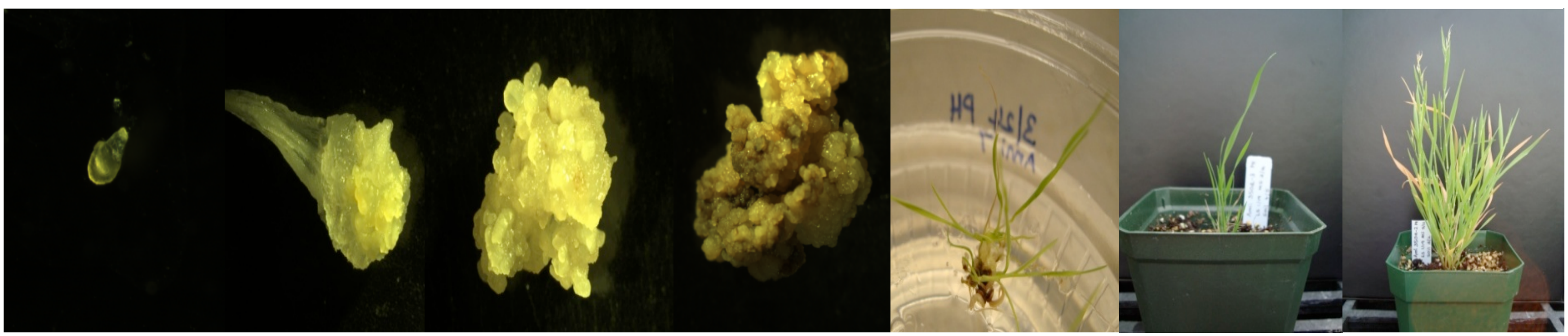
Arabidopsis thaliana *Brachypodium distachyon*

High efficiency brachy transformation

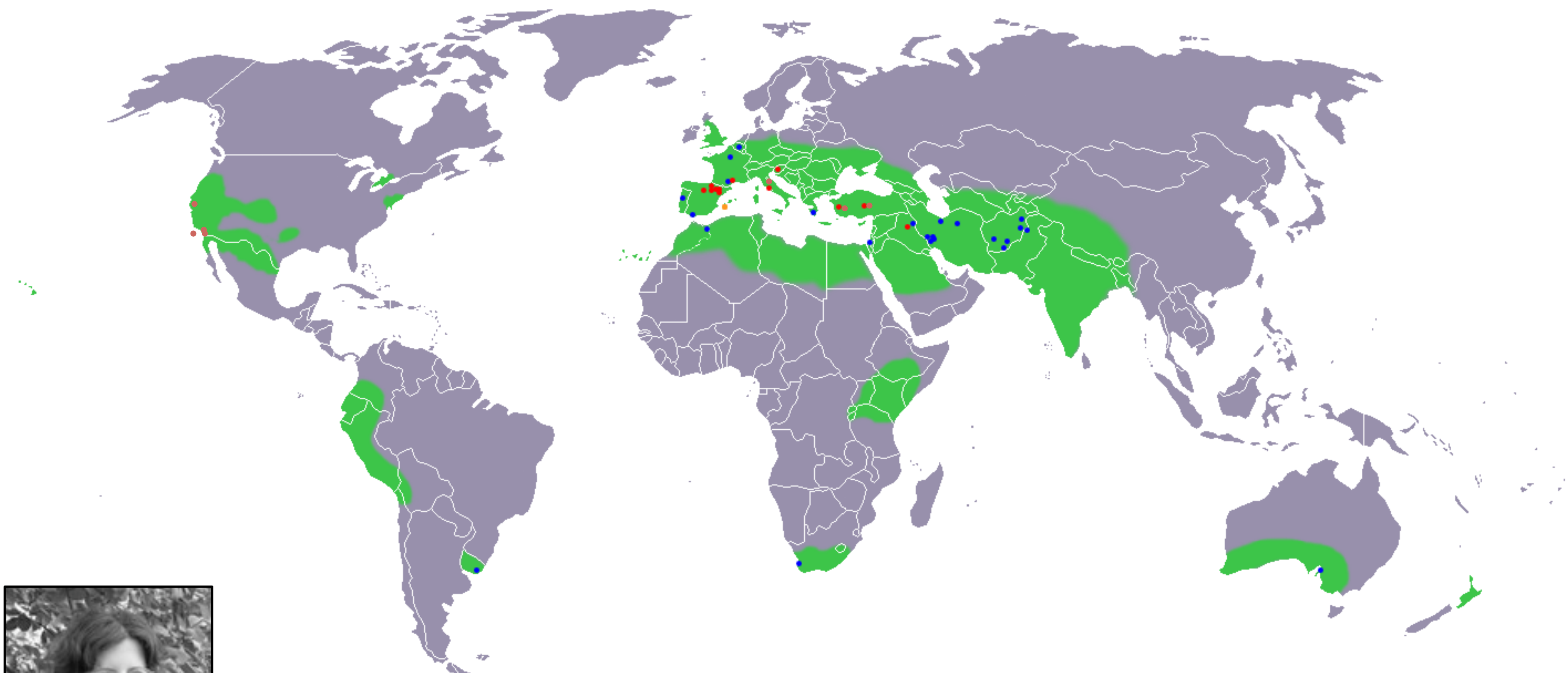
Transformation of Brachy calli

Regeneration of mutant plants

Analysis and characterization of the putative mutants



Brachypodium geographical distribution



Garvin et al, *Plant Genome* 2008

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- Seminars

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Faculty

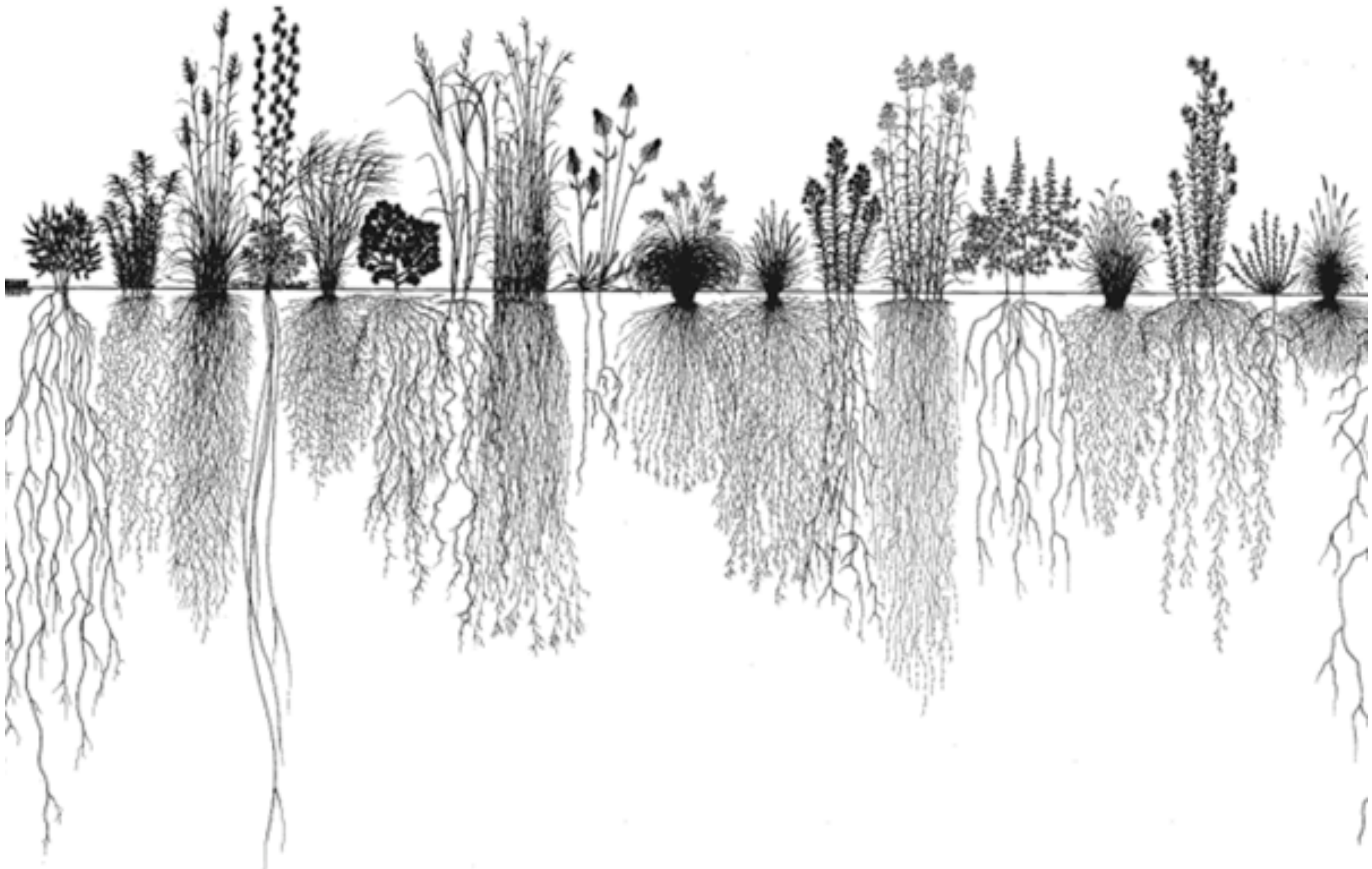
View

This special research initiative establishes a collaborative project employing Brachypodium to study plant and microbial sciences and related biofuel research. This has brought together Professors, Graduate Students and Researchers from departments ranging from Biology, Microbiology, Biochemistry and Molecular Biology (BMB), and Plant, Soil, and Insect, Sciences (PSIS) at UMass Amherst.

Contact information for these researchers is listed to the right.

Faculty

Dr. Tobias Baskin
Dr. Magdalena Bezanilla
Dr. Jeff Blanchard
Dr. Ana Caicedo
Dr. Michelle DaCosta
Dr. Samuel Hazen
Dr. Peter Hepler
Dr. Geunhwa Jung
Dr. Sue Leschine
Dr. Jennifer Normanly
Dr. Om Parkash
Dr. Danny Schnell
Dr. Elsbeth Walker



- Goal – low input production of high yielding dedicated energy crops with recalcitrant roots and stems highly amenable to biofuel conversion

Acknowledgements

- Li Lin
- Naomi Young
- Pubudu Handakumbura

- Pete MacAskill
- Rebecca Lamonthe
- Scott Lee
- Shan Chen
- Torie Carroll
- Gina Trabucco
- Mike Veling

- UN-L
  - Jeff Pedersen
- UMass
  - Sue Leschine
  - Tom Warnick
- UCSD
  - Steve Kay
  - Ghislain Breton

U.S. DEPARTMENT OF ENERGY